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13. ABSTRACT ***(Maximum 200 Words)***
The purpose of this project is to develop a diagnostic blood test for breast cancer. Specifically, we have proposed to test the feasibility of detecting breast cancer derived DNA shed into blood using a recently developed technique, methylation-specific PCR (MSP). The first aim of the project is to test breast tissue specimens and paired serum or plasma samples for patterns of DNA methylation using markers already known to be methylated in a high proportion of breast cancers. To this end, we have collected 55 sets of paired samples and we have tested 34 of the tumor samples for methylation of at least one marker. Based on our preliminary studies, it appears that the majority of breast cancers do have methylation of one or more of the genes that we plan to use for this phase of the project. While serum/plasma samples from breast cancer patients have not yet been tested, we have improved our technical ability to conduct MSP on DNA isolated from serum/plasma. The second aim of the project is to identify new sequences that are differentially methylated in breast cancers compared to normal tissues. Using methylation-specific arbitrarily primed PCR, we have identified one promising candidate sequence.

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Introduction

As outlined in the grant application, we expected the majority of our work during the first year to be devoted to *Task 1*: to test the ability of MSP to detect breast cancer derived DNA in plasma of breast cancer patients. We have progressed in this area by 1) collecting suitably matched plasma (or serum) samples and breast cancer tissue samples, 2) screening tumor samples for methylation of specific genes, and 3) optimizing protocols for measuring methylation of DNA derived from plasma (or serum). We have also progressed as planned in our work outlined as *Task 2*. For this task, we have performed AP-PCR on several breast cancer samples and we have isolated, cloned, and sequenced one DNA segment that is commonly differentially methylated between matched normal and tumor samples.

Body

Accomplishments, problems, and proposed solutions are described with reference to the tasks outlined in the Statement of Work:

Task 1: To test the ability of MSP to detect breast cancer derived DNA in plasma of breast cancer patients (months 1 – 36)

- **Test matched serum/plasma and tissue samples for methylation of *e-cadherin*, *GST*□, *ER*, and *HIC-1* (months 1 – 24)**

  Accomplishments: As indicated in the Statement of Work, our major effort in the first year of funding was directed toward these proof-of-principal studies. The first level of achievement toward accomplishing this goal is the collection of matched plasma/serum and tumor tissue samples. During the first year of funding, we have collected samples (matched plasma/serum and tissue) from 55 breast cancer patients.

  Serum/plasma samples are frequently limited in quantity and therefore it is essential to first screen tumor samples for methylation of specific genes before testing the serum/plasma samples. Using the methylation specific PCR reaction, we have tested 34 of the breast cancer samples and have found significant methylation of the 4 genes proposed for use in the pilot studies. These results are summarized in the table below.

  Methylation of genes in breast cancer samples

	e-cadherin	*GST π*	*ER*	*HIC-1*
Number of samples tested	34	34	20	20
Number of samples with methylation	18	14	11	17

  The limited quantity of serum/plasma samples also necessitates our optimizing protocols for DNA isolation, bisulfite treatment, and MSP. We have tested a number of variables for DNA isolation and bisulfite treatment and, although we have made significant improvements in out protocols, we believe

that testing of the serum/plasma samples should await further refinement of protocols. These issues are discussed further in the section below.
Problems: As discussed above, we continue to be concerned that treatment of sample DNA with bisulfite results in modified DNA of variable quality. This is a critical issue because many of the samples (particularly serum/plasma samples) are limited in quantity and have relatively low concentrations of DNA.
Proposed Solutions: We have empirically tested several methods for handling the DNA and have found that DNA purified by use of small purification columns (Quigen) after bisulfite treatment is improved with regard to ability to amplify. This work has been done on "spiked" serum from volunteers and will be now tested on serum and plasma from patients in the study.

- **Test matched serum/plasma and tissue samples for methylation of novel sites identified by work of task 2 (months 18 – 36)**
  We have not yet initiated work defined by this task.
- **Initiate additional studies of selected patient populations based on initial studies (months 24-36)**
  We have not yet initiated work defined by this task.

Task 2. To find novel aberrantly methylated sequences in breast cancers using methylation-specific arbitrarily primed PCR (months 6-30)

- **Perform AP-PCR on breast cancer samples (months 6-24)**
  Accomplishments: We have continued the AP-PCR work described as preliminary data for our grant application and we have repeatedly found a band suggestive of a sequence that is differentially methylated in breast cancers compared to normal tissues.
- **Isolate, clone, and sequence differentially methylated sequences from AP-PCR (months 12-30)**
  Accomplishments: The band described above has been isolated, cloned and sequenced. The sequence does not match any sequence deposited in the GenBank database. Further characterization of this methylated sequence will be conducted in subsequent years of this project.

Key Research Accomplishments

As described above, we have made progress in accordance with the schedule outlined in the Statement of Work. Although this progress will position us for testing the ability of the proposed strategy within the next year, we cannot yet report a definitive testing of our hypothesis at this time.

Reportable Outcomes

We have no reportable outcomes to date. As outlined in the Statement of Work, we expect reportable outcomes by the end of the second year of the project.

Conclusions

This project is on track to achieve the objectives as proposed. We are addressing some issues related to optimizing our methods for measuring DNA methylation in serum/plasma samples, and we expect to compare serum/plasma measurements to tissue measurements within the second year of the project. Although we have not yet discovered any useful novel aberrantly methylated sequences in breast cancers, we will continue to explore the approach outlined for the second task of the project.